

iBind™ Fluorescent Detection (FD) Solution Kit



Pub. Part No. 100022233 Pub. No. MAN0009825 Rev. D.0

Package Contents	Cat. No. SLF1019 Size 10 rxns <ul style="list-style-type: none">▪ iBind™ FD 5X Buffer▪ iBind™ 100X Additive▪ iBind™ FD 10% SDS
Storage Conditions	<ul style="list-style-type: none">▪ Store buffer and additive at 4°C (do not freeze)▪ Store SDS at room temperature
Required Materials	<ul style="list-style-type: none">▪ iBind™ Window Cover (or aluminum foil)▪ iBind™ Device▪ iBind™ Card▪ Blotting roller▪ Alexa Fluor® 680 or IRDye® 680LT Goat Anti-Rabbit▪ Alexa Fluor® 790 or IRDye® 800CW Goat Anti-Mouse
Timing	Preparation time: ~10 minutes Run time: Allow well 4 to empty; ~2.5 hours or longer
Product Description	<ul style="list-style-type: none">▪ This iBind™ Fluorescent Detection (FD) Solution Kit facilitates primary and secondary antibody binding of membrane-bound proteins in western detection.▪ This kit is compatible with Alexa Fluor® and/or IRDye® conjugated secondary antibodies.
Important Guidelines	<ul style="list-style-type: none">▪ Wear gloves when handling the iBind™ card.▪ Do not open the iBind™ device after closing it over an iBind™ card.▪ Make sure iBind™ FD 10% SDS is completely in solution before use. SDS is added to the secondary antibody solution to decrease background for both PVDF and nitrocellulose membranes.▪ Protect the device from light with the iBind™ window cover or aluminum foil.
Online Resources	Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support .



iBind™ Fluorescent Detection (FD) Solution Kit

Prepare Antibodies

Dilute antibodies with 1X iBind™ FD Solution. Each blot requires 2 mL of each antibody solution.

Component	Primary Antibody (1° Ab) Solution
1X iBind™ FD Solution	2 mL
Primary Antibody	Final antibody concentration equal to the manufacturer's recommended dilution
Component	Secondary Antibody (2° Ab) Solution
1X iBind™ FD Solution	2 mL
iBind™ FD 10% SDS	10 µL
▪ Alexa Fluor® 680 OR ▪ IRDye® 680LT	▪ 1 µL (1:2000 dilution) ▪ 0.5 µL (1:4000 dilution)
▪ Alexa Fluor® 790 OR ▪ IRDye® 800CW	▪ 1 µL (1:2000 dilution) ▪ 0.67 µL (1:3000 dilution)

Limited Product Warranty

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iBind™ Fluorescent Detection (FD) Solution Kit

iBind™ Fluorescent Detection Solution Kit Protocol

Follow the diagram below to prepare solutions and membrane and perform antibody binding with the iBind™ device.

Timeline	Steps	Procedure Details												
1 	Prepare 1X FD Solution	Standard 1X iBind™ FD Solution is recommended for most primary antibodies. Use the Optional solution only if initial results give low sensitivity.												
		<table border="1"> <thead> <tr> <th rowspan="2">Component</th> <th colspan="2">1X iBind™ FD Solution (for 1 mini blot)</th> </tr> <tr> <th>Standard</th> <th>Optional</th> </tr> </thead> <tbody> <tr> <td>iBind™ FD 5X Buffer</td> <td>6 mL</td> <td>1.5 mL</td> </tr> <tr> <td>iBind™ 100X Additive</td> <td>75 µL</td> <td>300 µL</td> </tr> <tr> <td>Distilled water</td> <td>23.9 mL</td> <td>28.2 mL</td> </tr> </tbody> </table>	Component	1X iBind™ FD Solution (for 1 mini blot)		Standard	Optional	iBind™ FD 5X Buffer	6 mL	1.5 mL	iBind™ 100X Additive	75 µL	300 µL	Distilled water
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2 	Prepare diluted antibodies	See "Prepare Antibodies" for details.												
3 	Load iBind™ card and membrane in iBind™ device	<ol style="list-style-type: none"> Immerse the membrane in 5 mL of 1X iBind™ FD Solution. Place the iBind™ card on the iBind™ device, making sure the card is aligned with the stoppers. Evenly apply 5 mL of 1X iBind™ FD Solution to the card so that the Flow Region is completely wet. Add 1 mL 1X iBind™ FD Solution to the center of the Membrane Region. Place the blot on the card, protein side down, with the low MW region toward the stack. Remove any air bubbles between the iBind™ card and the membrane with a blotting roller. 												
4 	Add prepared antibody and wash solutions	<table border="1"> <thead> <tr> <th>iBind™ Device Well Number</th> <th>Solution</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>2 mL diluted primary antibody</td> </tr> <tr> <td>2</td> <td>2 mL 1X iBind™ FD Solution</td> </tr> <tr> <td>3</td> <td>2 mL diluted secondary antibody</td> </tr> <tr> <td>4</td> <td>6 mL 1X iBind™ FD Solution</td> </tr> </tbody> </table>	iBind™ Device Well Number	Solution	1	2 mL diluted primary antibody	2	2 mL 1X iBind™ FD Solution	3	2 mL diluted secondary antibody	4	6 mL 1X iBind™ FD Solution		
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5 	Run	<p>Close the iBind™ well cover to minimize evaporation. Cover the viewing area with the iBind™ window cover or aluminum foil.</p> <p>Leave the device undisturbed until the last well (Well 4) is empty, ~2.5 hours or longer.</p>												
6 	Detect blot	<p>Rinse the membrane in distilled water.</p> <p>Scan wet or dry membranes on the LI-COR® Odyssey® CLx scanner according to manufacturer recommendations.</p>												

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